Ultrasonics 78 (2017) 146–151

Contents lists available at ScienceDirect

Ultrasonics

journal homepage: www.elsevier.com/locate/ultras

Ultrasonic method for monitoring the clotting process during whole blood coagulation

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ARTICLE INFO

Article history:
Received 31 October 2016
Received in revised form 21 February 2017
Accepted 21 February 2017
Available online 24 February 2017

Keywords:
Ultrasonic measurement
Ultrasound velocity
Blood clotting
Clot structure
Acrylamide polymerisation

ABSTRACT

The purpose of this work was to develop a multichannel ultrasonic measurement method for monitoring a spatially non-uniform blood clotting process. This novel method is based on simultaneous multi-channel measurements of ultrasound propagation velocities in different horizontal cross-sections of clotting blood.

The most common method used for determining blood-clotting time is the capillary tube method. For this purpose ultrasonic methods based on measurements of the velocities of ultrasound waves in clotting blood are also used. Measurement results essentially depend on the propagation path of the ultrasonic wave in a blood sample. The ultrasound velocity changes as fresh blood transforms into clot plus serum. The objective of this work was to develop a measurement method that allows one to measure ultrasound velocity and its evolution in time and space in an evolving clot while avoiding the influence of serum.

To achieve this objective, a novel method has been proposed that is based on ultrasound propagation velocity measurements in different horizontal cross-sections of clotting blood using a pulse-echo mode. Such a technique enables researchers to monitor the clotting process and a clot's spatial structure, which are different in different layers due to the influence of gravity. The four-channel measurement chamber utilizing this method has been designed and manufactured. For the generation and reception of ultrasonic waves of high frequency, wide band (3–20 MHz at −6 dB) ultrasonic transducers were developed. To verify that the multi-channel measurement system was operational, a special procedure based on monitoring of a polymerisation process in the acrylamide solution was proposed.

Performance of the developed method was investigated by measuring clotting blood (sample volumes of less than 0.6 ml) at the frequency of 12 MHz. The results revealed that a clot structure indeed varies within a blood sample due to the influence of gravity; clotting times are different in different horizontal layers of the clot and range from 9 to 15 min, defined by the standard capillary method. Clotting times are determined precisely from abrupt increases in ultrasound velocity. Uncertainty of the ultrasound velocity measurements was less than ±0.05 m/s. The experiments were performed at 36.90 ± 0.01 °C.

The proposed method may be exploited for monitoring polymerisation reactions in the chemistry field, as well.

1. Introduction

The purpose of this work was to develop a multichannel ultrasonic measurement method for monitoring a spatially non-uniform blood clotting process. This novel method is based on simultaneous multi-channel measurements of ultrasound propagation velocities in different horizontal cross-sections of clotting blood.

Till now various ultrasonic methods have been used for blood coagulation studies, such as ultrasound spectroscopy [1], measurements of ultrasound wave velocity and attenuation [2–8], scattering [2,3,5,6,8,9], acoustic radiation force [10], impedance [11] as well as shear wave technique [11]. Recent detailed reviews discuss *in vitro* and *in vivo* applications of ultrasound in blood coagulation [12,13]. A main purpose of all these investigations was measurement of blood clotting time. There are various standard non-ultrasonic methods for determining clotting time, the most common of which being the capillary tube method. Usually, the observed clotting time is 5–15 min. Various standard methods give slightly different clotting time values [14]. Evidently, this may be the case for ultrasonic methods as well. On the other hand, the

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http://dx.doi.org/10.1016/j.ultras.2017.02.017
0041-624X/

coagulation process is spatially non-uniform due to influence of gravity, but this effect has not been analysed in the majority of previous works.

For evaluation of blood clotting time, we have developed a method that is based on the measurement of ultrasound velocity variations in a whole blood small sample (<1 ml) as fresh blood transforms into clot plus serum [15]. We found that serum forms above the clot in a measurement chamber due to gravity. Therefore, the measurement results essentially depend on a propagation path of the ultrasonic wave in the blood sample, as the clotting process is spatially non-uniform. Usually, ultrasonic transducers used for generation and reception of ultrasonic waves are mounted at the bottom or the top of the measurement chamber. In this case, the ultrasonic wave travels through both the clot and serum; therefore, variations of the ultrasound velocity are caused not only by changes in the structure of the clot but also by variations in the serum properties [16]. The variations in serum properties affect ultrasound velocity variations in clotting blood, frequently masking the end of a clotting process.

During clotting, blood structure varies in volume and time. Because blood corpuscles are influenced by gravity, clot structure should be different at different distances from the bottom of a blood sample. These differences can be detected by ultrasound. Indeed, in recent studies it has been found that gravity exhibits influence on microscopic structures as small as a human cell. Thus, gravitational forces might have an effect on blood clotting, especially on the action of the thrombocytes that participate in blood clotting [17].

The first quantitative results showing structure variations in volume and in time were described in our previous investigations [18–20]. Those experiments were performed in a through-transmission mode [19]. The influence of gravity on measurement results was investigated by changing the orientation of the measurement chamber from vertical to horizontal. Obviously, the influence of gravity on the results in both orientations was quite different and clotting curves obtained by ultrasonic measurements were different depending on the channel's orientation. Analysis of the results showed that a novel measuring method and a more sophisticated geometry of the measurement chamber are necessary in order to monitor the blood clotting process across its spatial and time domains.

The objective of this work was the development of such a measurement method, allowing for the measurement of ultrasound velocity variations in an evolving clot while taking into account the spatial non-uniformity of the clotting process and avoiding the disturbing influences of serum.

The proposed ultrasonic measurement method and the developed multi-channel measurement chamber are described in the second chapter. In the third chapter a dynamic verification method of the measurement system is presented. Investigation of the performance of the developed method is described in the fourth chapter.

2. Measurement principle

To achieve the above objective, a novel method based on multi-channel measurements of ultrasound propagation velocities in different horizontal cross sections of a clotting blood using a pulse-echo mode is proposed. We designed and manufactured the four-channel measurement chamber implementing this method (Fig. 1).

The measurement chamber has a rectangular cross section with dimensions 3.5 mm × 7 mm. The height of the chamber is 25 mm, what means that the blood quantity necessary for obtaining measurements is less than 0.6 ml. The chamber is immersed in a water bath with a temperature kept at 36.90 ± 0.01 °C. The chamber was made of copper. Copper was selected as it has good thermal properties and provides good and fast temperature stabilization of the blood sample. The internal surface of it was coated by gold for biocompatibility.

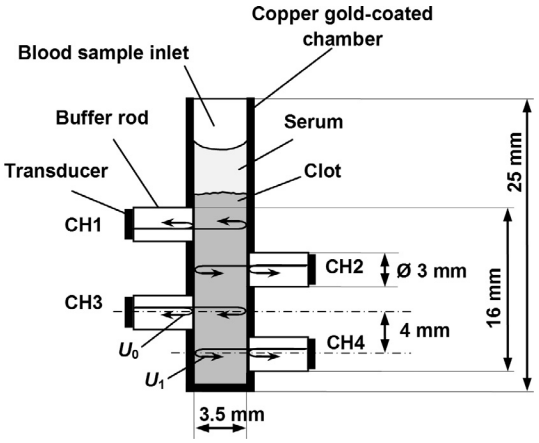

Fig. 1. Four-channel measurement chamber: 1, 2, 3 and 4 measurement channels.

The number of measurement channels was based on many considerations. To minimize the volume of the blood sample used for measurements, the dimensions of the measurement chamber were chosen to be as small as possible: 3.4 mm × 7 mm × 25 mm. The number of the measurement channels is defined by the lateral dimensions of the measurement channels, which are defined by the diameters of PMMA buffer rods. To radiate a plane wave, the diameter of the buffer rod should be at least 10 wavelengths. In our case, the wavelength in a blood sample at the frequency of 12.5 MHz was $\lambda = 0.22$ mm; therefore, the rod diameter d was selected 3 mm, and $d/\lambda = 13.6$ and was more than required. To reduce the crosstalk between neighbouring channels, a gap of 1 mm was left and even and odd channels were placed on opposite sides of the chamber. It means that one channel needs 4 mm along the measurement chamber. Four measurement channels occupy 16 mm along the chamber. A small space on the top of the chamber the length of which is 9 mm is left for injection of a blood sample by a syringe.

Ultrasonic transducers with PMMA type plastic (Plexiglas®) buffer rods are made of Pz29 piezoelectric ceramics (Ferroperm Piezoceramics A/S, Hejreskovvej 18A, DK-3490 Kvistgaard, Denmark) with a resonance frequency of 12.5 MHz. Such a high operational frequency was chosen in order to obtain the necessary accuracy of ultrasound velocity measurements. We have selected the piezoelectric ceramic Pz29 for ultrasonic transducers because it is usually recommended for medical transducers by the manufacturer and possesses a high electromechanical coupling coefficient for thickness mode $k_t = 0.52$. The pulse and frequency responses of the transducers were measured in a pulse-echo mode and are presented in Fig. 2, which shows that they possess 140% bandwidth at the −6 dB level. These responses were measured in distilled water.

As it was mentioned above the ultrasound velocity is measured by a pulse-echo method which is based on measurement of the ultrasound impulse propagation time in a blood sample. The measurements were performed under assumption that in a blood sample a plane ultrasonic wave propagates. Deviations from the plane wave may cause diffraction errors. To reduce such errors, we proposed use of additional buffer rods between piezoelectric elements and the measurement chamber [21]. Due to the buffer rods, ultrasound velocity measurements are performed not in a near field but